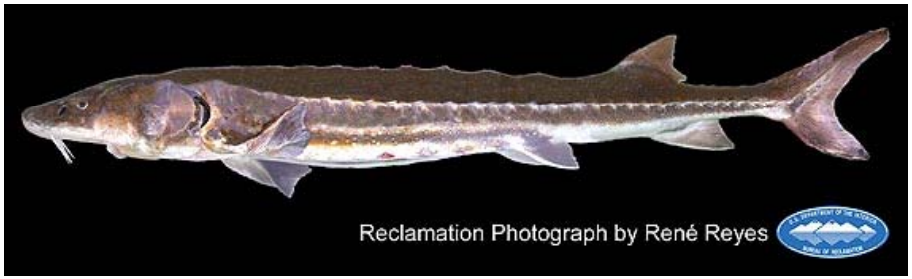
# Applications of Environmental DNA (eDNA) Methodology

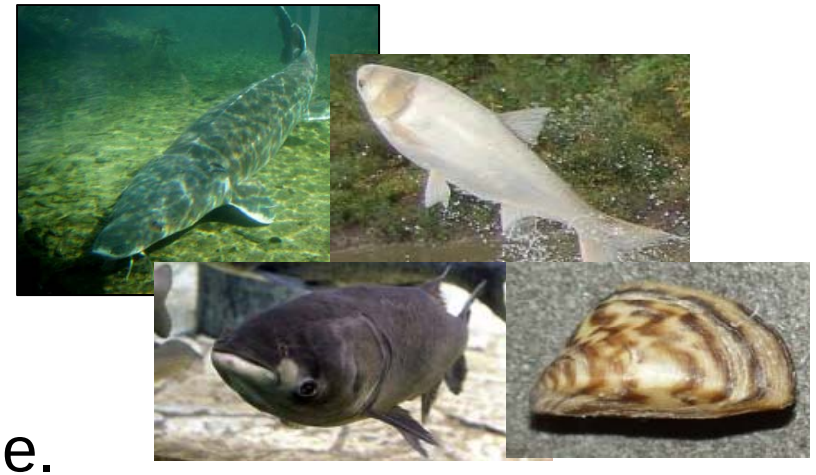



**Project Lead:** Richard Lance

**Project Team*:*** Heather Farrington, Eliecer Navarro, Xin Guan, Matthew Carr

# Introduction

- Accurate monitoring of aquatic species in environmental systems can be difficult
- Current survey methods may not always provide accurate assessments of target species presence or distribution when species are rare or elusive
- Environmental DNA (eDNA) is useful in the monitoring of invasive or imperiled species at early stages of invasion, rare, or when elusive.
- May be useful for other purposes, even when species are common and otherwise observable.



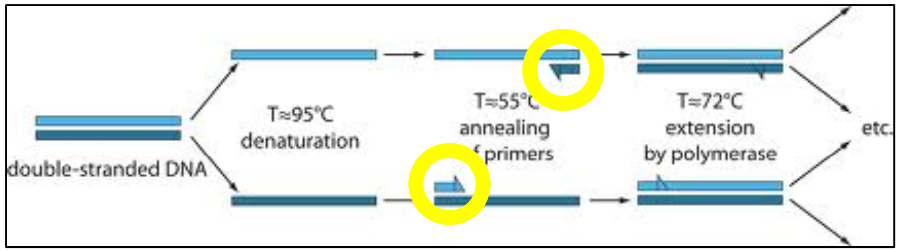
# eDNA

- Biological materials shed into an aquatic system can be detected in filtrate from water samples.

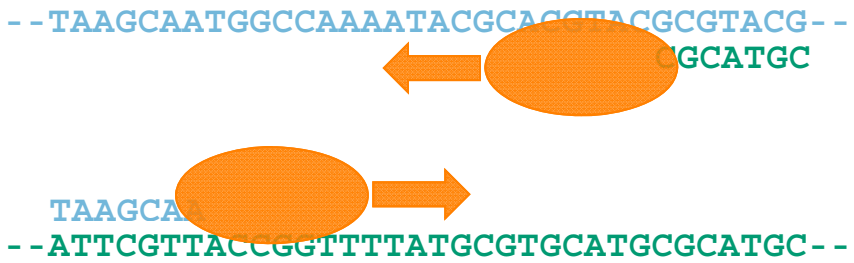
- DNA sources include
  - Epithelia (scales)
  - Cells excreted with urine and feces
  - Blood or tissues from injuries
  - Reproductive cells (sperm, eggs)

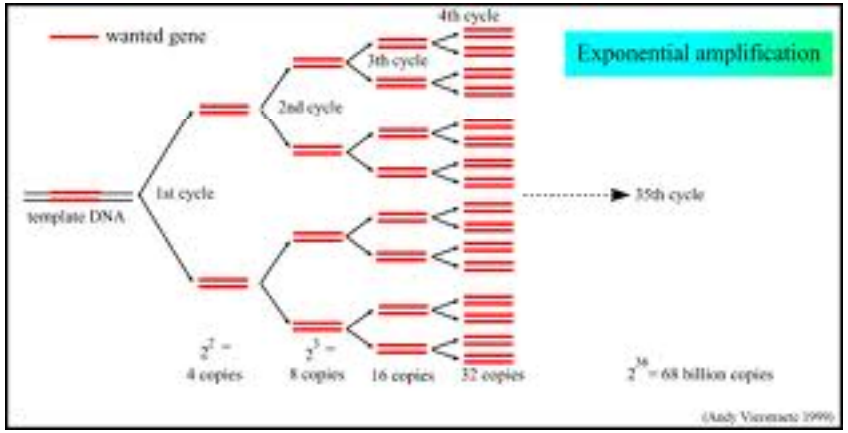
# Getting Started

- The utility of eDNA methodology is dependent upon the quality of species-specific primers (“markers”) designed for the target organism




PCR



# Getting Started

Appropriate eDNA markers should be:

**Accurate**: Always detect individuals of target species and do not cross-react with non-target species

**Sensitive**: Provide detections when only small amounts of DNA in water

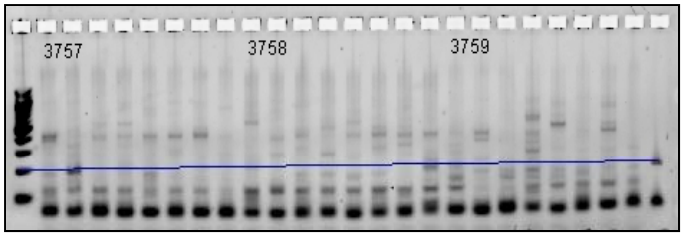
**Robust**: Have relatively high viability and detectability under environmental conditions

# General Method

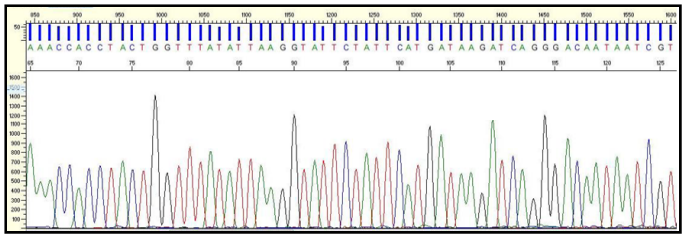
- Water samples collected and filtered to capture any suspended cells or tissues
  - Filters trap organic material from numerous species
  - Non-DNA molecules are removed from filtered material and DNA is purified using commercial kits

# General Method

- Polymerase Chain Reaction (PCR) amplifies target species DNA from environmental samples containing DNA from multiple organisms
- DNA fragments are visualized using gel electrophoresis

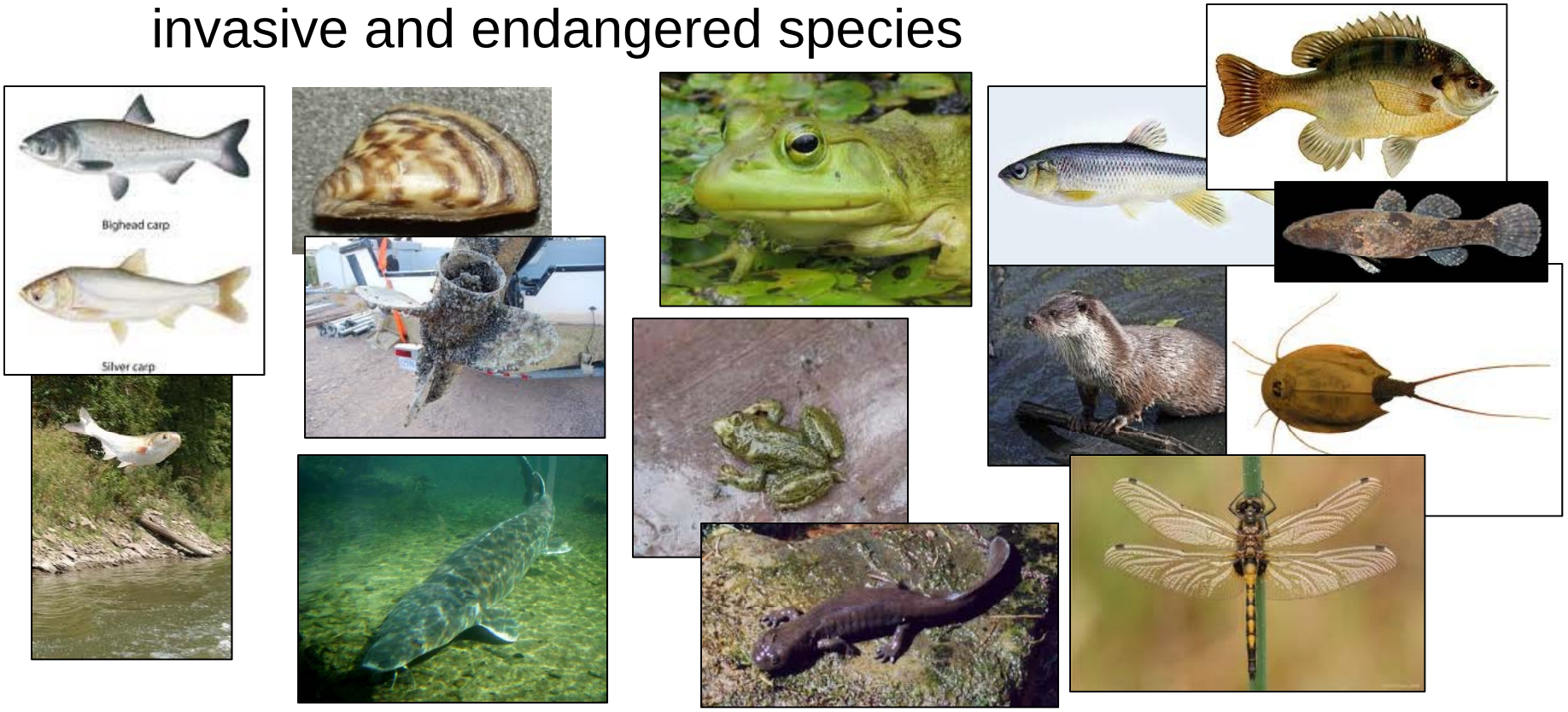



- Fragments of expected size are sequenced and compared to known target sequences for verification of species identity



# Current Applications

- eDNA methods are currently being used and developed in a variety of systems to monitor both invasive and endangered species



# Asian Carp Detection

- eDNA is involved in the monitoring efforts for invasive Asian Carp Species (BigHead and Silver) within the Chicago Area Waterway System (CAWS)

Bighead carp

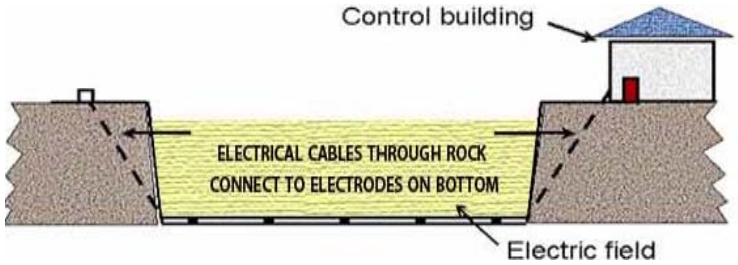
- Bighead Carp (*Hypophthalmichthys nobilis*)

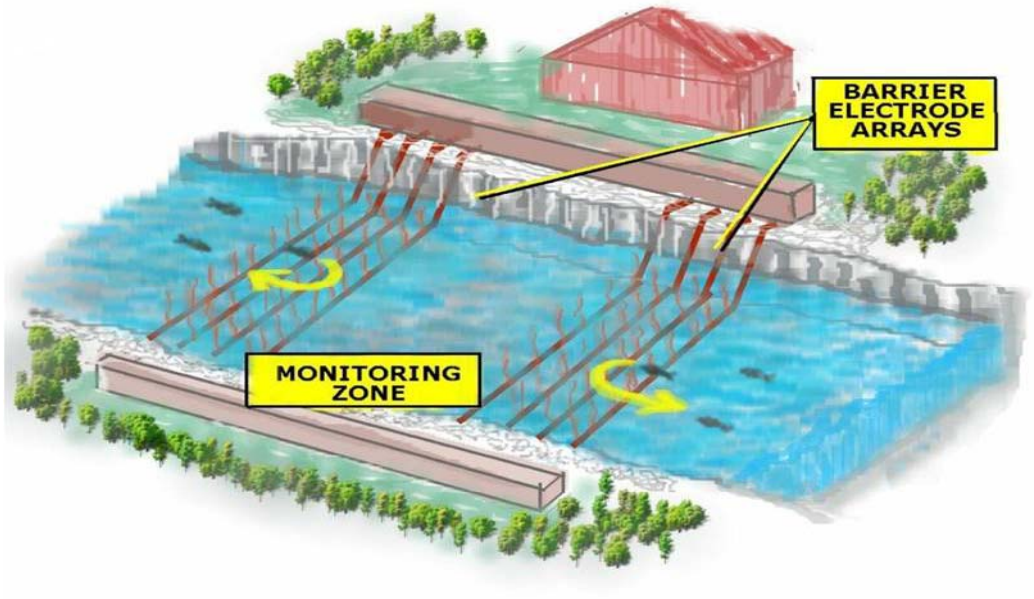
Silver carp

- Silver Carp (*Hypophthalmichthys molitrix*)
- Serious concerns about the impact of these species in the Great Lakes

# Asian Carp Detection

- Study Area: Chicago Area Waterway System
- Sampling Objectives:
  - Monitor the possible presence of Asian carp above and below electrical Aquatic Nuisance Species Dispersal Barrier
  - Results used to direct action plans for more extensive field sampling and eradication decisions



# eDNA Calibration Study (eCALS)

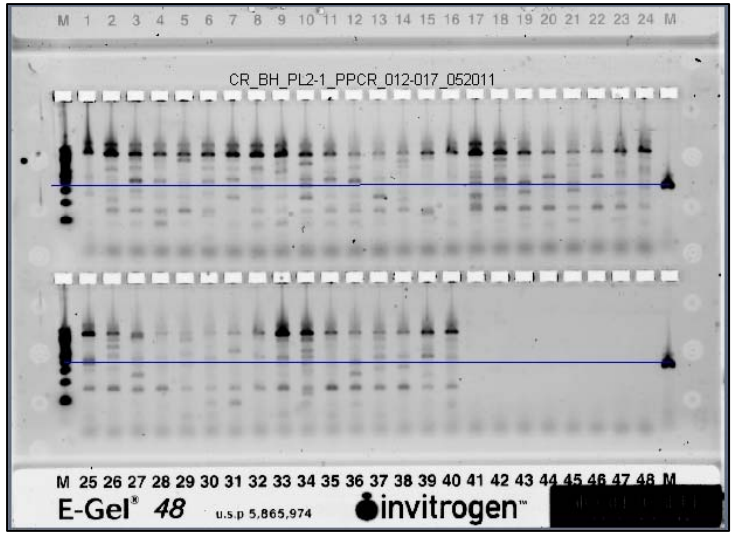
- Conducted in support of Asian Carp project
- eDNA calibration studies are underway to examine
  - Various sampling methods (volume, filter material, etc.)
  - eDNA degradation under various conditions (temp, pH, etc.)
  - eDNA loading rates from different sources, biomasses
  - Potential vectors of eDNA introduction to system

# eDNA Calibration Study (eCALS)

- USACE, ESGS, USFWS collaboration funded by EPA
  - Increased efficiency and statistical power
    - New Markers & High Throughput
  - Increasing inferential power
    - Alternative vectors, eDNA loading and degradation
    - Spatial Models
    - Risk and Decision Analysis

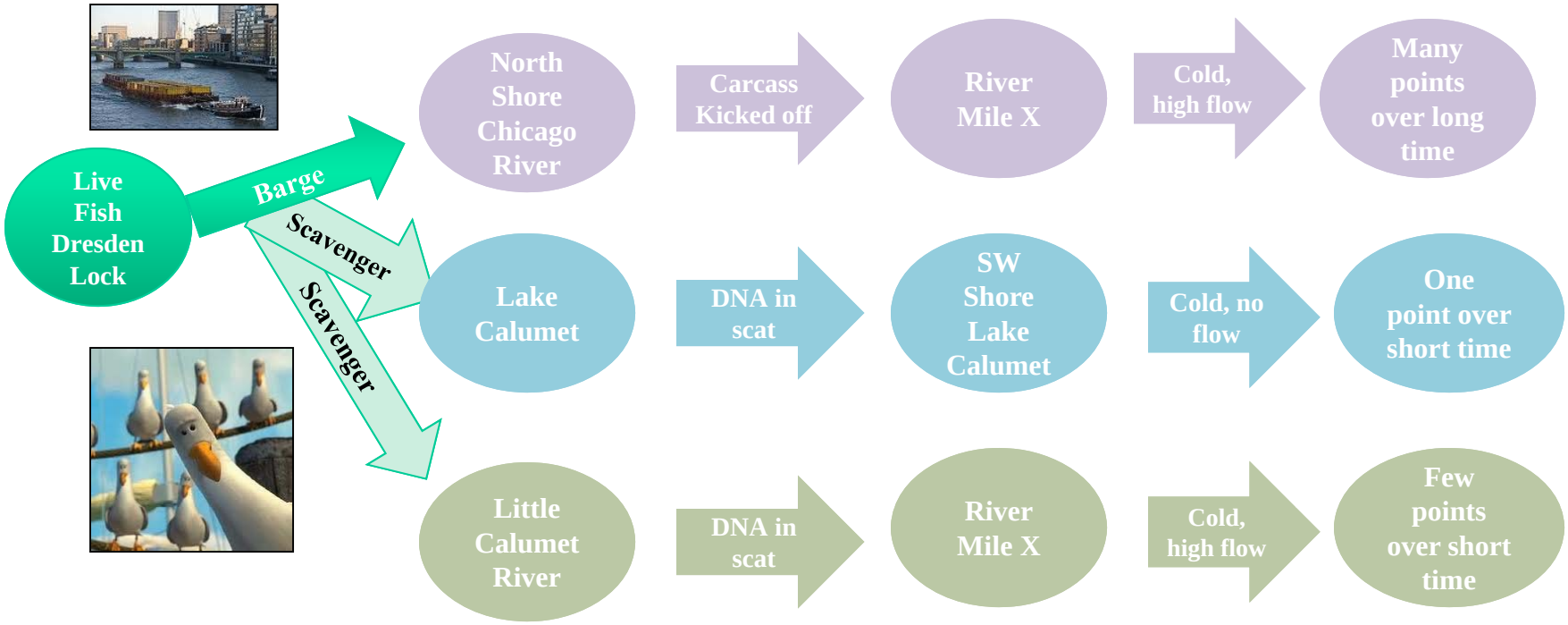
# eCALS
## New Markers & Throughput

- Ways to make eDNA assays more efficient and cost-effective
  - Streamlining of sample collection and processing
  - Faster DNA extraction methods
  - Develop more informative, more efficient markers
    - qPCR
    - Next-generation sequencing



# eCALS
## Alternative vectors & eDNA degradation


## Understanding the Dynamics of eDNA Detections
### *eDNA Mobility Conceptual Terminology & Example*



# eCALS
## Alternative vectors & eDNA degradation


**Origin** → **Vectors** → **Receiving Point**

| | |
|---|---|
| Fish Markets & CSOs | Bait Trade |
| Fertilizer | Flooding (ponds, Des Plains) |
| Birds & Other Predators | Gear contamination |
| Barges (carcasses) | Dredging |
| Animal Feed | Barges & Boats (slime on hull) |

**Release Modes** → **Release Point** → **Environmental Factors** → **Positive Detection Point**

**DNA loading** from:
Live fish
Fish carcasses
Predator feces
Sediment

**Degradation** from:
Temperature
TOC
pH
dO
UV light
Microbial Activity

# Dreissenid Mussel Detection

- September 2010 started looking at the possible use of eDNA methodology for invasive Dreissenid mussels
- Zebra Mussel (*Dreissena polymorpha)*
- Quagga Mussel (*Dreissena bugensis*)
- Invasiveness is enhanced by
  - Prolific Breeding Rate
  - Free-Floating Veligers
  - Ability to attach to solid substrata

# Dreissenid Mussel Detection

- Study Area: Lake Pepin, Wisconsin
- Goals of study:
  - Apply current eDNA protocol to the detection of zebra mussel veligers
  - Examine the potential use of DNA-binding dyes in discriminating between DNA from live vs. dead organisms.
  - Compare current surface sampling to alternative sieve cloth sampling
- Sample number and overall sample range were limited due to the considerable amount of time required for pumping, but eliminates the need for laboratory filtration

# Dreissenid Mussel Detection

- Use of DNA-binding dyes looked promising, but were inconclusive
  - Additional studies need to be done.
- Sieve sampling 75% better than surface sampling
- Sieve method has potential if efficiency could be improved

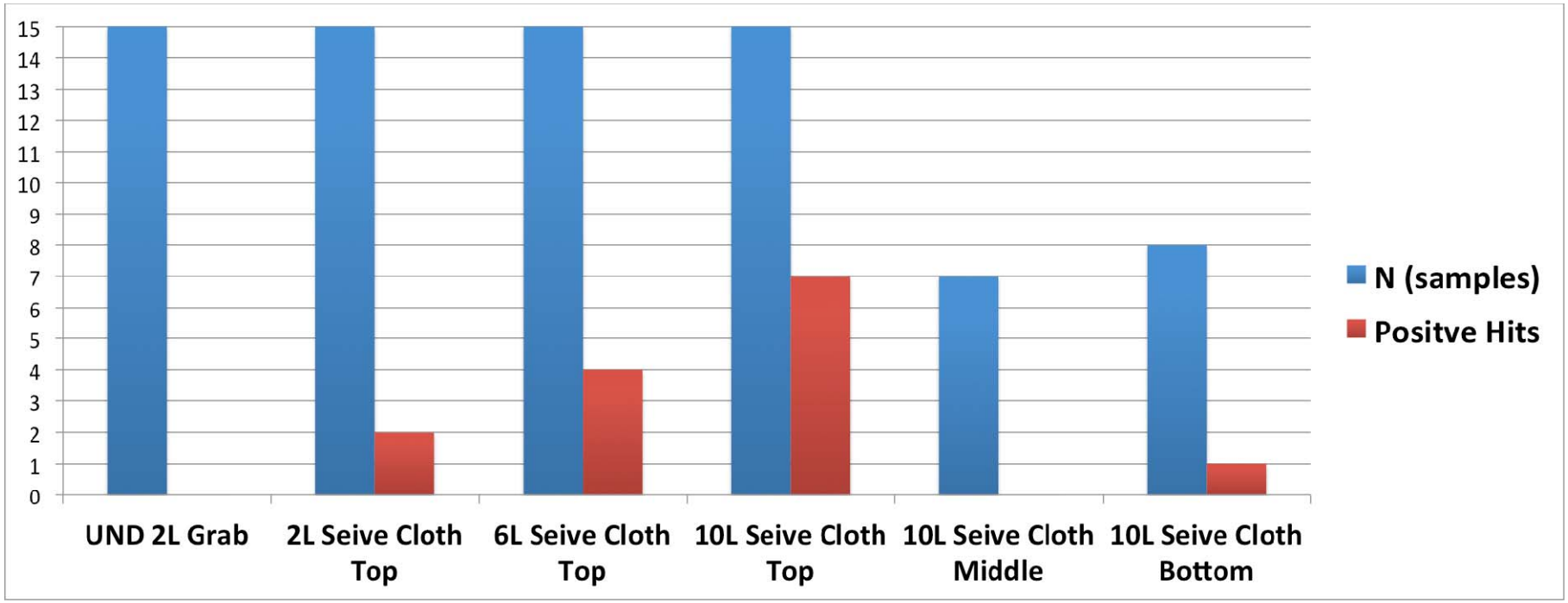
# Method Optimization

## eCALS

- Testing alternative sampling protocols
  - Standard “grab” sample and filtration
  - Sieve cloth sample
    - Combines collection and filtration into single step
    - Allows filtering of larger volumes of water
- Testing differences in detection success with varying sample volumes

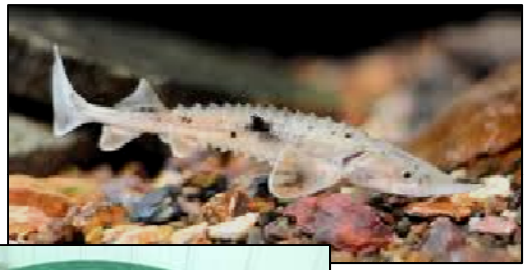
# Method Optimization

## eCALS



# Detection and Monitoring of Sturgeon

- eDNA monitoring is particularly well-suited for sturgeon
  - Relatively rare
  - Bottom dwellers
  - Often anadromous (seasonal migrations)



- Can be used to:
  - Monitor seasonal movements
  - Identify habitat use
  - Determine environmental windows
  - Direct restocking and conservation efforts

# Sturgeon Assay Development

- **Genetic Markers:**
  - 8 potential genetic markers identified for detection of Acipenseridae
  - Successfully tested markers in 7 of 9 North American sturgeon

✓ Lake (*Acipenser fulvescens)*
✓ Gulf (*A. oxyrinchus desotoi)*
✓ White (*A. transmontanus)*
✓*Green (A. medirostris)*
✓ Pallid (*Scaphirhynchus albus)*
✓ Shovelnose (*S. platorynchus)*
✓ Alabama (*S. suttkusi)*

• Shortnose (*A. brevirostrum)*
• Atlantic (*A. oxyrinchus oxyrinchus)*

# Sturgeon Assay Development

- Largely successful testing of markers against non-target species (ongoing)

  Sunfish (pumpkinseed, green, orange-spotted), Shiner (emerald, spotfin, golden), Bass (white, smallmouth, largemouth), Perch (white, yellow), Carp (mirror, common, grass, silver, bighead), Crappie (white, black), Buffalo (black, smallmouth), Catfish (channel, flathead), Quillback, Freshwater Drum, Brown Bullhead, Bluegill, Gizzard Shad, Round Goby, Brook Silverside, Goldfish, Bluntnose Minnow

- Paddlefish can be a problem
- Sensitivity limited in some regions due to paddlefish cross-reaction

# Sturgeon Assay Development



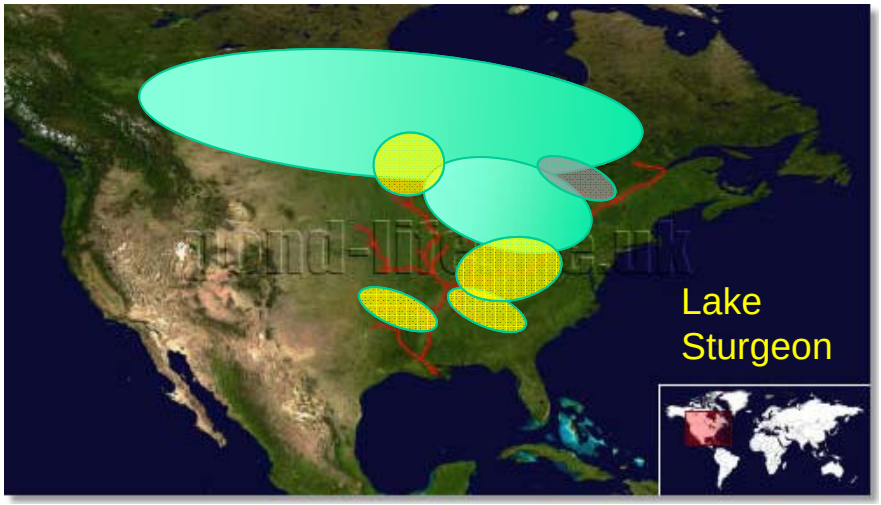
Colored ovals showing geographic ranges where the Acipenseridae eDNA marker can be used to detect single species (i.e. sites where only 1 species occurs).

Blue = any of 8 markers should work, based on current testing

Yellow = any of 3 markers should work, based on current testing; markers less sensitive to paddlefish

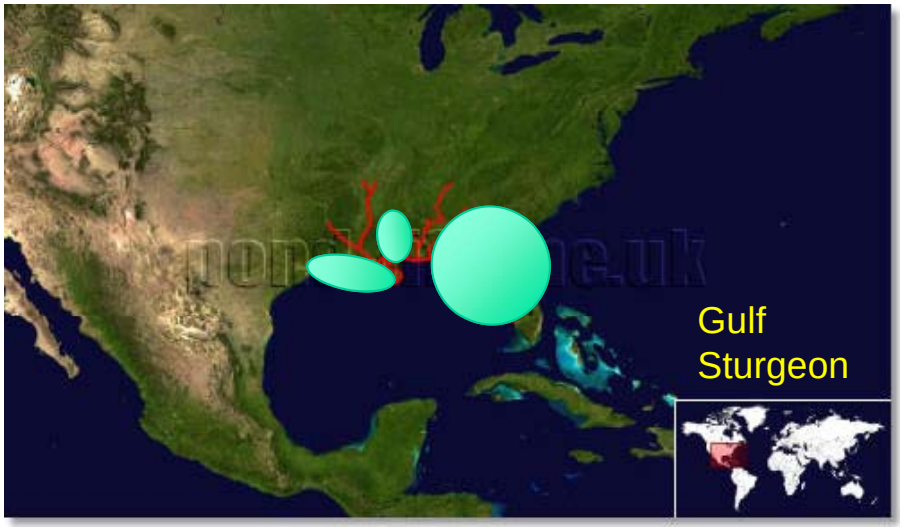
DNA sequencing can also discriminate between species in some cases

# Sturgeon Assay Development

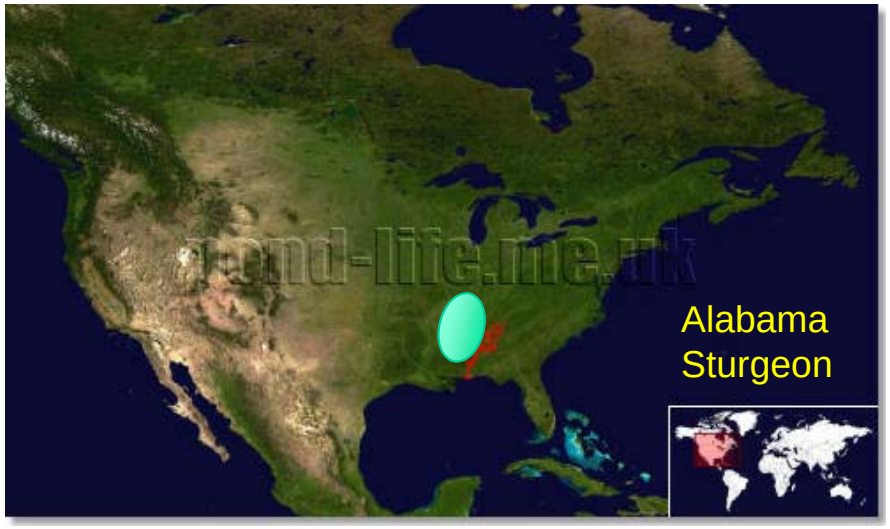





# Sturgeon Assay Development





# Sturgeon Assay Development

In areas of sturgeon species overlap –

- Blocking primers
  - *Acipenser* vs. *Scaphirhynchus*
    - Gulf Sturgeon vs. Alabama Sturgeon started
  - *Acipenser* vs. *Acipenser*
    - White Sturgeon vs. Green Sturgeon started
  - Sturgeon vs. Paddlefish
- New markers
  - *Scaphirhynchus* vs. *Scaphirhynchus*
    - Shovelnose vs. Pallid

| | |
|---|---|
| Alabama Sturgeon 2 | TATCATCCTAGCCCTATGGGGCATTATTATGACAGGATCGATCTGTTTACGACAGACAGACCTAAAA |
| Alabama Sturgeon 1 | TATCATCCTAGCCCTATGGGGCATTATTATGACAGGATCGATCTGTTTACGACAGACAGACCTAAAA |
| Gulf Sturgeon 4 | TATCATCCTGGCCCTATGGGGCATCATCATAACCGGGTCAATTTGCCTCCGACAAACAGACCTAAAA |
| Gulf Sturgeon 5 | TATCATCCTGGCCCTATGGGGCATCATCATAACCGGGTCAATTTGCCTCCGACAAACAGACCTAAAA |

# Uses for eDNA

- Effective and cost-efficient means for detecting presence of eDNA (putative presence)
  - Estimated cost for processing sample:
    - Carp (current): $175
    - Sturgeon (optimized, optimistic): $60
- Can be used to guide standard sampling, monitoring, or stocking efforts